Protecting Effect of Antioxidants (Antox) on the Dentate Gyrus of Aged Male Albino Rat

[1]Mohammed. H. Mohammed and [2]Mostafa A. Abouelnaga

[1]Department of Anatomy and Embryology, Faculty of Medicine, Assiut University and
[2]Faculty of Medicine, AL- Azhar University– Damietia branch

Abstract: Introduction: Aging has been defined as a time-related loss of the capacity of cells to maintain their functions. A primary cause of the aging process has been claimed to be the reduced antioxidant defense.**Aim of the study:** This study aims to elucidate the histological features of the dentate gyrus in male albino rats during aging process and to investigate the important protective role of antioxidant (Antox) on the aging of the dentate gyrus of adult male albino rats. **Material and methods :**41 male adult albino rats were used and were classified into three groups; Control adult group (3-6 months), Control aged group which was further classified into three subgroups aged 12, 18, and 24 months, respectively and Antox -treated aged group which was also classified into three subgroups aged 12,18 and 24 months. The treated animals received Antox dissolved in water at a dose level of 3.4 mg/kg body weight 3 times weekly for 3 months starting at 9,15 and 21months for the three age subgroups respectively. Sections of hippocampus were prepared were prepared for light and electron microscope examinations. **Results:** With light and electron microscope, there was a progressive increase in the appearance of dark neurons with advancing age in addition to a noticeable neuronal loss and a decrease in the frequency of appearance of neurons in the control aged groups. Senile changes such as the accumulation of lipofuscinin the neuronal perikarya and changes in the nucleus, mitochondria, rough reticulum, and *Golgi* apparatus were observed in the control group. Also, membrane-bound organelle-free areas were observed. Degenerated neurons, with shrunken nuclei and ill-defined few cytoplasmic organelles, were observed with advancing age. After the treatment with Antox, the senile changes were less when compared with the control aged group. It was also noticed that, the Antoximproving age - associated histological changes were best especially at the middle age (12 months) rather than that of the early old age (18 months) and the oldest age (24 months), mostly due to the irreversible degenerative changes which had occurred at older age groups prior to the treatment with Antox.**Conclusion:** The present study showed that the age - associated histological changes may be the basis for the age associated functional changes of the gyrusof the hippocampal formation, which may be manifest in elderly people by disturbances in motor coordination and declines.The present study also demonstrates the effectiveness of the combination antioxidants (Antox) in reducing the age-related histological changes in the dentate gyrus of the hippocampal formation. So, it is recommended to investigate its use in age-related neurodegenerative

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1. Introduction

The dentate gyrus (DG) is a simple cortical region that is an integral portion of the larger functional brain system called the hippocampal formation **(Kempermann, 2002).** The hippocampus is a neural structure in the medial temporal lobe that has a distinctive curved S shape. The hippocampal formation is defined as the complex of six structures: Gyrusdentatus, hippocampus proprius, subiculumproprium, presubiculum, parasubiculum and area entorhinalis **(Andersen et *al.*, 2000).**

The DG consists of three layers: the outer molecular layer, the middle granule cell layer and the inner polymorphic layer (hilus). The principal neurons of the DG are the granule cells, which have most of the features typical of small neurons. They are organized compactly and form an inferior and superior blade in the rat DG **(Laatsch and Cowan, 1966)**. The hilar cells resemble the morphology of the spiny pyramidal neuron **(Seroogy*et al.*, 1983**). Experimental studies proved that the hippocampus has a very important role in the process of learning and in a wide range of memory types particularly in spatial memory **(El Falougy and Benuska, 2006).**

The hippocampal dentate gyrus was reported to be one of the few regions of the mammalian brain where neurogenesis continue to occur throughout adulthood. The neurogenesis in the dentate gyrus was thought to play an important role in hippocampus-dependent learning and memory **(Gao, *et al.*, 2007, and Li, *et al.*, 2008).** Hence, DG is thought to contribute to new memories as well as other functional roles **(Saab *et al.*, 2009)**. Aging is characterized by a general decline in physiological function that leads to morbidity and mortality **(Miyoshi, *et al.*, 2006).**

Hippocampal circuitry is particularly vulnerable to aging and neurodegenerative conditions

(Chohan*et al.*, 2009). Normal aging brings with it changes in dopaminergic and memory functions **(Morcom*et al.*, 2010).** Moreover, aging is the greatest environmental risk factor for the sporadic cases of Alzheimer's disease (AD) which is progressive neurodegenerative disorder characterized by a progressive memory loss and cognitive decline **(Caselli, *et al.*, 2006).**

The antioxidants are either reactive chemicals such as Antox or specialized enzymes as catalase. The body produces enzymatic antioxidants but cannot make the antioxidant chemicals (e.g. Antox, C and flavonoids), that protect the sites in the body that the enzymatic antioxidants cannot reach **(Hagga*et al*,. 2006; Mosad*et al.*, 2007)** mentioned that beta carotene (a carotenoid metabolic precursor to vitamin A), Antox, vitamin C and selenium are involved in the overall cellular antioxidant defense mechanism.

Antioxidents nutrients, as ascorbic acid, tocopherol, B-carotene, etc., are considered to give protection against oxidative damage induced by different toxicants and reduce the activity of free radical-induced reactions **(McCall and Balz, 1999)**. Antox is an antioxidant drug composed of selenium, vitamin A acetate, ascorbic acid and vitamin E. Antox was used in therapy of different liver diseases **(Wastone*t al.*, 1999, Hamooda, *et al.*,2003,Zhong and Lemasters, 2004 and Oz *et al.*, 2004).**

The aim of the study

The goal of the present study is to elucidate the histological features of the dentate gyrus in male albino rats during aging process and to investigate the important protective role of antioxidant (Antox) on the aging of the dentate gyrus of adult male albino rats.

2. Material and Methods

Material:

A total number of 41 male albino rats (average weight 200-250 gm) were used in this study. The animals were isolated in clean properly ventilated cages in the animal house of AssiutUniversity under normal conditions with an appropriate temperature, normal light and dark cycle and free access to food and water.

The animals were divided into 3 groups:

Group I (Control adult group): This group includes 5 male rats aged 3-6 months.

Group II (Control aged group):This group includes 18 male rats that were equally divided according to their age (Shettyand Turner, 1999) and its corresponding human age (Flood, et al., 1987) into three subgroups:

Subgroup II a: 12 months-aged control animals were used representing the middle age group which corresponds to the age of fifties in human.

Subgroup II b: 18 months-aged control animals were used representing the early old age group that corresponds to the age of seventies in human.

Subgroup II c: 24 months-aged control animals were used representing the oldest old age group that corresponds to the age of nineties in human.

Subgroup III (Antox-treated aged group): This group includes 18 male rats that were equally divided into three subgroups:

Subgroup III a 12 months-aged Antox-treated animals,

Subgroup III b: 18 months-aged Antox-treated animals,

Subgroup III c: 24 months-aged Antox-treated animals.

Drug dosage and administration:

The treated animals received Antox dissolved in water at a dose level of 3.4 mg/kg body weight 3 times weekly for 3 months starting at 9,15 and 21months for the three age subgroups respectively. Antox tablets composed of selenium, medicinal yeast, ascorbic acid, vitamin A acetate and vitamin E (Hawazen and Maisaa,2007).

Methods:

Tissue Preparation

Each animal was anaesthetized with ether, its heart was exposed, and saline solution was perfused through the left ventricle until the coming fluid was blood-free. Then perfusion was done with Bouin's fixative for light microscopy, and with cold 4% gluteraldhyde in a buffered cacodylate solution pH 7.4 for electron microscopy. The cranial cavity was opened; the brain was carefully dissected out and left immersed in the fixative and undisturbed for one hour. Then, the right cerebral hemisphere was sectioned coronallyfor studying the right dentate gyrus of the hippocampal formation.

For light microscopy, paraffin sections (5-7µm) of tissue specimens were prepared and stained with Harris haematoxylin and eosin according toDrury and Wallington (1980). Semithin sections (0.5-lµm) of the specimens fixed in 4% gluteraldhyde were stained with toluidine blue (Gupta, 1983) and were examined with light microscope. Subsequently, thin sections (500-800A) were obtained for the selected areas in semithin sections, contrasted with uranyl acetate and lead citrate (Reynolds, 1963), and studied with the transmission electron microscope, JEOL (J.E.M.- 100 CXI 1) and photographed at 80 K.V. in Assiut University Electron Microscope Unit.

3. Results

A- With light Microscope:

Group I (Control adult group): Plate 1

The haematoxylin and eosin stained sections of the dentate gyrus of control adult male albino rats show that the dentate gyrus forms a distinctive V-shaped structure that surrounds the free border of the Amon's horn (Plate 1, Fig. 1). The dentate gyrus consists of three layers, an outer molecular layer, a

central granule cell layer, and an inner polymorphic layer (Plate 1, Fig. 2). The molecular layer is formed mainly of nerve fibers and few nerve cells. The granule cell layer contains the perikarya of granule cells, which are densely packed with little or no intervening tissue. The nuclei of granule cells are rounded or oval in shape and pale basophilic in stain. Their nucleoli are densely stained basophilic and usually peripherally located. A thin rim of cytoplasm is surrounding the nucleus of granule cells (Plate 1, Fig. 3).

The semithin sections stained with toluidine blue confirm that the dentate gyrus is formed of three layers, the outer molecular layer, the granule cell layer and the inner polymorphic layer (Plate 1, Fig. 4). The outer molecular layer consists mainly of nerve fibers together with few scattered cells. The middle granule cell layer shows dense packing of the granule bodies with little intervening tissue. The granule cells usually assume liar or polyhedral shape. Their nuclei appear pale, rounded or ovoid in with single dense peripheral nucleoli and finely dispersed chromatin. Nuclei are surrounded by thin run of pale staining cytoplasm (Plate 1, Fig.5). The inner polymorphic layer shows a variety of cells of different types. These cells include large pyramidal neurons, few displaced granule and some glial cells (Plate 1, Fig. 6).

Group II (Control aged group):Plate 2

The haematoxylin and eosin-stained sections of the dentate gyrus of the control 12 months - aged male albino rats show some changes in the shape and staining intensity of granule neurons mainly in the deeper parts of the granule cell layer. The cell bodies of these neurons assume irregular profiles with irregular outline and show increase staining intensity. Their nuclei are irregular in shape and deeply stained with no visible nucleoli. The more superficial neurons show normal shape and staining intensity with rounded pale nuclei and visible dense nucleoli (Plate 2, Fig. 1).

For control 18 months – aged group, the heamatoxylin and eosin – stained sections, the granule cell layer of dentate gyrus of male albino rats shows that many granule cell bodies appear darkly stained with irregular outline. Their nuclei are irregular, deeply stained and surrounded by thin rim of deeply stained cytoplasm. Some of the granule cell bodies appear relatively normal shape and staining intensity (Plate 2, Fig. 2).

For control 24 months – aged group, the heamatoxylin and eosin – stained sections in the dentate gyrus of male albino rats show that the most of the granule neurons appear darkly stained with irregular outline and deeply stained nuclei and cytoplasm. Empty spaces are observed among the granule cell bodies (Plate 2, Fig.3).

The semithin sections of the dentate gyrus of control 12 months – aged group show deeply stained granule cell bodies especially in the more basal parts granule cell layer. The nuclei of these neurons appear darkly stained with hardly visible deeply stained nucleoli and surrounded by thin rim of stained cytoplasm, which shows small vacuoles. Other granule cell bodies show pale stained, clear, vacuolated cytoplasm and their nuclei relatively normal in shape and staining intensity. The remaining cells show more or less normal shape and staining intensity of their nuclei and cytoplasm (Plate 2, Fig. 4).

For control 18 months – aged group, the semithin sections of the dentate gyrusshow that the granule cell layer contains many darkly stained granule cell bodies having irregular outlines. The nuclei of these dark cells appear darkly stained with hardly visible, darkly stained nucleoli. Their cytoplasm is darkly stained forms thin rim around the nuclei. Some of the granule neurons show relatively normal staining of their nuclei, while, their cytoplasm contains 56 pale vacuolated areas (Plate 2, Fig. 5).

For control 24 months – aged group, the semithin sections of the dentate gyrusshow that the granule cell layer is mainly formed of the darkly stained granule cells which have deeply stained irregular nuclei surrounded by thin rim of darkly stained cytoplasm. Few of the granule cells appear pale stained. Their nuclei are relatively normal in staining intensity. Their cytoplasm appears pale vacuolated (Plate 2, Fig.6).

Group III (Antox treated aged group): Plate 3

The haematoxylin and eosin-stained sections in the dentate gyrus of Antox-treated 12 months-aged group, animals show that most of the granule cell bodies in the granule cell layer are almost of normal shape and staining intensity. They have rounded pale nuclei with visible dense nucleoli and surrounded by thin rim of cytoplasm. Only few granule cell bodies in the basal part of the granule cell layer appear darkly stained with deeply stained nuclei and cytoplasm (Plate 3, Fig. 1).

For treated 18months-aged group, the haematoxylin and eosin stain, sections in the dentate gyrus of Antox-treated male albino rats show that many granule ells are relatively normal in shape and staining intensity. They have rounded or oval pale nuclei with visible dense nucleoli and surrounded by thin layer of cytoplasm. Few granule neurons, located basely in the granule cell layer, appear darkly stained with irregular outline. These dark cells are comparatively much less frequent than those observed m the control of the same age group (Plate 3, Fig. 2).

For treated 24 months-aged group, the haematoxylin and eosin-stained sections in the dentate gyrus of Antox-treated male albino rats show that some of the granule cell bodies have rounded or

oval large pale nuclei with visible dense nucleoli and surrounded by pale staining cytoplasm. The other granule cell bodies appear darkly stained nuclei and cytoplasm. No empty spaces are observed among the granule cells in comparison with the control group of the same age (Plate 3, Fig. 3).

In semithin sections of the dentate gyrus stained with toluidine blue, the granule cell layer of 12 months-aged group, is mainly formed of pale staining cells. These cells show rounded or oval pale nuclei with visible dense nucleoli and surrounded by pale staining cytoplasm which are more or less similar to those of the control adult age group. Only few granule cell bodies appear darkly stained and have irregular outline, deeply stained nuclei and thin rim darkly stained cytoplasm. These dark cells are observed in the basal part the granule cell layer and are less than those observed in the control 12 months-aged animals (Plate 3, Fig. 4).

For treated 18 months-aged group, semithin sections in the dentate gyrus stained with toluidine blue confirm that many granule cell bodies have large oval or rounded pale nuclei with visible dense nucleoli and surrounded by pale staining cytoplasm. Small pale vacuolated areas are observed infrequently in the cytoplasm of these pale staining granule cells. Few granule cells appear darkly stained and are comparatively much less frequent than those served in the control of the same age group (Plate 3, Fig. 5).

For treated 24 months-aged group, the semithin sections in the dentate gyrus of this group stained with toluidine blue show that some of the granule cell bodies are much similar to those of the control adult age group. Some granule cells have relatively normal nuclei surrounded by lightly stained, vacuolated cytoplasm, and other granule cells are darkly stained with irregular outline and deeply stained nuclei and cytoplasm. The frequency of these dark granule cells is muchless in comparison with the control group of the same age (Plate 3, Fig. 6).

B- With Electron Microscope:

Group I (Control adult group): Plate 4

The dentate gyrus shows the detailed fine structure of its three layers. The molecular layer is formed mainly of unmyelinated nerve and few myelinated fibers having variable size and containing neurofibrils and mitochondria. Few displaced granule cells are seen especially in the inner half of the molecular layer near to the granule cell layer. They resemble all the features of the ordinary granule cells; their nucleus is large and nearly rounded with finely dispersed chromatin and dense nucleolus. Scattered glial cells are seen in this layer with small nuclei containing dense clumps of chromatin and surrounded by scanty amount of cytoplasm (Plate 4, Fig. 1).

Also, the granule cell layer shows the characteristic close packing of cell bodies of the granule neurons with little intervening tissue. The cell bodies of the granule neurons appear nearly circular or polygonal in shape. Their nuclei are oval rounded with uniformly dispersed chromatin and single electron dense peripheral nucleoli. The cytoplasm forms a narrow rim around the nucleus and contains cisternae and vesicles of Golgi apparatus, mitochondria, ribosomes and few short cisternae of rER (Plate 4, Fig. 2).

In the polymorphic layer, the most striking feature is the presence of large numbers of myelinated nerve fibers especially immediately beneath granule cell layer. Also, unmyelinated nerve fibers can be seen in this layer. Few g1ial cells are also observed with small nucleus containing clumps of dense chromatin and surrounded by scanty amount of cytoplasm (Plate 4, Fig. 3).

Group II (Control aged group): Plate 4

The dentate gyrus of control 12 months – aged male albino rats shows that some of the granule cell bodies are more electron dense in their cytoplasm and nuclei. Their nuclei appear indented or irregular in shape. Their cytoplasm shows dilated short segments of rER. Some of the granule neurons show relatively normal shape and electron density of their nuclei, but the cytoplasm of these neurons contains accumulations of lipofuscin pigment, membrane-bound vacuoles and few dilated rER segments. In the cytoplasm of both the dark and pale granule cells, some mitochondria appear distorted (Plate 4, Fig. 4).

The dentate gyrus of control 18 months – aged male albino rats shows that many granule neurons are irregular in outline and their cytoplasm and nuclei appear dark. The cytoplasm of these cells show distorted Golgi cisternae, distorted mitochondria and dilated rER cisternae. Some of the dark granule neurons appear degenerated with dark homogenous (amalgamated) nuclei and few distorted cytoplasmic organelles. Some of the granule neurons show relatively normal electron density of their nuclei. Some lipofuscinpigment bodies, dilated rER segments and membrane- bound vacuoles are present The neuropil between the granule cell bodies shows many organelles – free areas (Plate 4, Fig. 5).

The dentate gyrus of control 24 months – aged male albino rats shows that most of the granule neurons appear dark (more electron dense) with irregular outline, markedly electron dense nuclei, no visible nucleoli. The cytoplasm of these cells show distorted Golgi cisternae, distorted mitochondria and fragmented short segments of rER and lipofuscin pigment. Few of the granule cells have relatively normal electron density of their nuclei with wide organelle – free areas and marked distortion of the mitochondria in their cytoplasm (Plate 4, Fig. 6).

Group III (Antox treated aged group): Plate 5

The dentate gyrus of Antox-treated 12 months-aged male albino shows that most of the granule cells appear relatively similar to those control adult group. They contain large rounded pale nuclei having dispersed chromatin and electron dense nucleoli. The nuclei of these are surrounded by thin rim of cytoplasm that shows relatively electron density and normal appearance of the neuronal organelles mitochondria, andrER. No membrane-bound or organelle-free areas are observed in their cytoplasm (Plate 5**,** Fig. 1).

The dentate gyrus of Antox-treated 18 months-aged male albino rats shows that many granule neurons have large rounded pale nuclei with finely dispersed chromatin. The cytoplasm of these neurons is of normal electron density and shows normal appearance of most neuronal organelles. Some of the granule cells show dilated rER cisternae with little or no distortion of the mitochondria (Plate 5, Fig. 2). Infrequently, small organelle-free areas are served in the cytoplasm of few granule cells. Only few granule cells appear dark electron dense similar to those in the control group of the same age (Plate 5, Fig. 3).

The dentate gyrus of Antox-treated 24 months-aged male albino rats showed that some of the granule cells had rounded or oval pale nuclei with finely dispersed chromatin and electron-dense nucleoli. The cytoplasm neurons appeared relatively of normal electron density with more normal neuronal organelles. The cytoplasm of other granule cells dilated rER segments, small lipofuscin pigment, some mitochondria and organelle-free areas. Some of the neurons appeared dark with irregular outline and more electron- dense nuclei and cytoplasm (Plate 5, Fig. 4).

4. Discussion

Dentate gyrus is a subregion of the hippocampus that is crucial in cognitive functions such as learning and memory (Tashiro *et al.,* 2007). Brain aging is the key risk factor for the development of cognitive impairment and the development of age related degenerative pathologies (Brayne, 2007). The dentate gyrus is differentially vulnerable to the aging process (Small *et al.,* 2004).

In the present study, male albino rats were utilized to avoid the female hormonal effect. That was supported by previous investigator who suggested that estrogen enhanced cell proliferation during proestrus resulted in more immature neurons in the hippocampal formation of females compared with males and present the possibility that these new cells exert an important influence on hippocampal function (Tanapat*et al.,*1999 and 2001).

In the current study, by studying the dentate gyrus of control aged animals, dark neurons appeared in the granule cell layer which increased in frequency progressively with increasing age. These dark granule neurons appeared shrunken with irregular outline and increased staining intensity, dilated perinuclear cisterna and rER cisternae, together with distorted mitochondria and *Golgi* cisternae in their dark electron dense cytoplasm. These age-associated nuclear alterations **are in** agreement with the s**tudies** of **Radak, *et al.,* (2006)** who **found** an increase of oxidative DNA damage in neurons of regions involved in neurodegenerative diseases, such as Alzheimer's disease, which is suggestive of an accelerated aging process in specific populations of neurons.

The progressive accumulation of mitochondrial dysfunction has been proposed to contribute to the neuronal death and dysfunction with aging. The decline in neuronal metabolism is reported to be a constant alteration in the old brain as a whole, and discrete zones of the CNS are particularly disposed to develop alterations in neuronal metabolic efficiency with aging, including the hippocampal formation. In addition, there was a noticeable accumulation of lipofuscin pigment in the granule neurons of the dentate gyrus of aged animals which increased progressively with advancing age. The age - related accumulation of lipofuscin pigment bodies observed in the present study is in accord of several experimental studies which demonstrated that the most persistent age-related cytological change is the deposition of lipofuscin pigment bodies m the neurons of the hippocampal formation which increases in size and complexity with increasing age **(Sushma, *et al.,* 2011).**

The organelle-free areas observed in the present study are in agreement with the studies of **Sushma, *et al.,* (2011**) who described extreme cytoplasmic vacuolation in the aged hippocampal cells and reported that these vacuolated cells may be regarded as the initial stages of necrotic cell death. A more progressive increase in neuronal cell death by apoptosis has been reported in neurodegenerative disorders, such as Alzheimer's disease **(Ivins, *et al.,* 2000).**

It has been reported that when loss of neurons occurs asin sequence of aging, the healthy neurons in the same area can assume parallel functions to maintain functional stability and, later on, as loss of neurons progresses over the compensatory mechanisms of the surviving neurons, the function is definitely disturbed. It is reasonable, to consider that the changes occurring in the histological structure of the dentate gyrus during aging may be mediated by damage after decline of the antioxidant defense system **(Small *et al.,* 2004).**

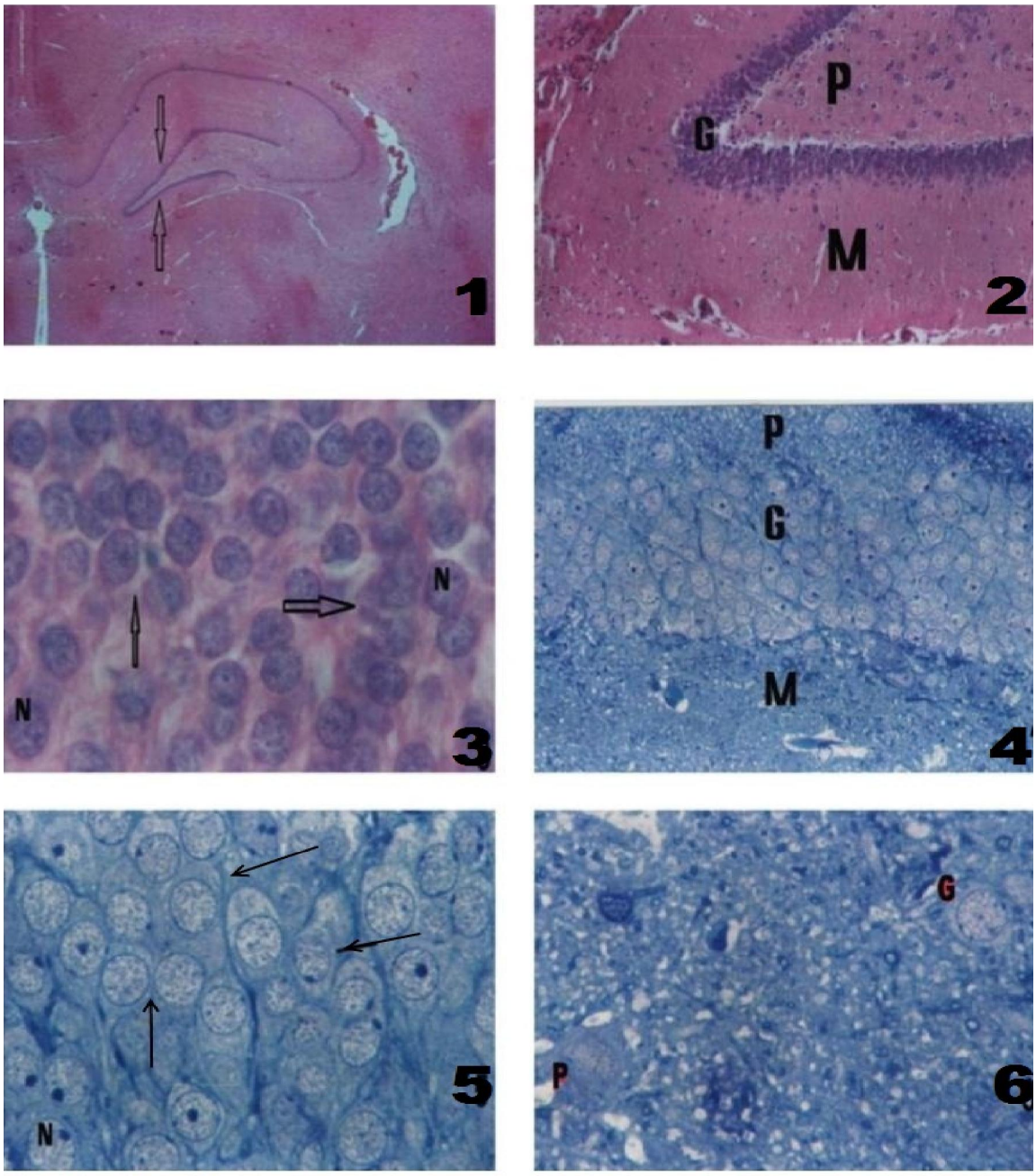

PLATE 1

Fig. 1: A photomicrograph of a section in the hippocampal formation of the control adult group showing; the V –shaped dentate gyrus (arrows) H.&E. X 100

Fig. 2: A photomicrograph of a section in dentate gyrus of the control adult group showing; the outer molecular layer (M), the middle granular layer (G) and the inner polymorphic layer (P). H.&E. X 400

Fig. 3: A photomicrograph of a section in dentate gyrus of the control adult group showing; the close packing of granule cells(arrows) and their large, pale rounded nuclei (N). H.&E. X1000

Fig. 4: A photomicrograph of a semithin section in dentate gyrus of the control adult group showing; the outer molecular layer (M), the middle granular layer (G) and the inner polymorphic layer (P). Toluidine blue X400

Fig. 5: A photomicrograph of a semithin section in dentate gyrus of the control adult group showing; the densely packed granule cells(arrows) with rounded paledense nuclei and thin rim of cytoplasm around the nuclei (N).Toluidine blue X1000

Fig. 6: A photomicrograph of a semithin section in dentate gyrus of the control adult group showing; apart of the polymorphic layer with scattered pyramidal cells (P),granule cells (G) and nerve fibers in between. Toluidine blue X1000

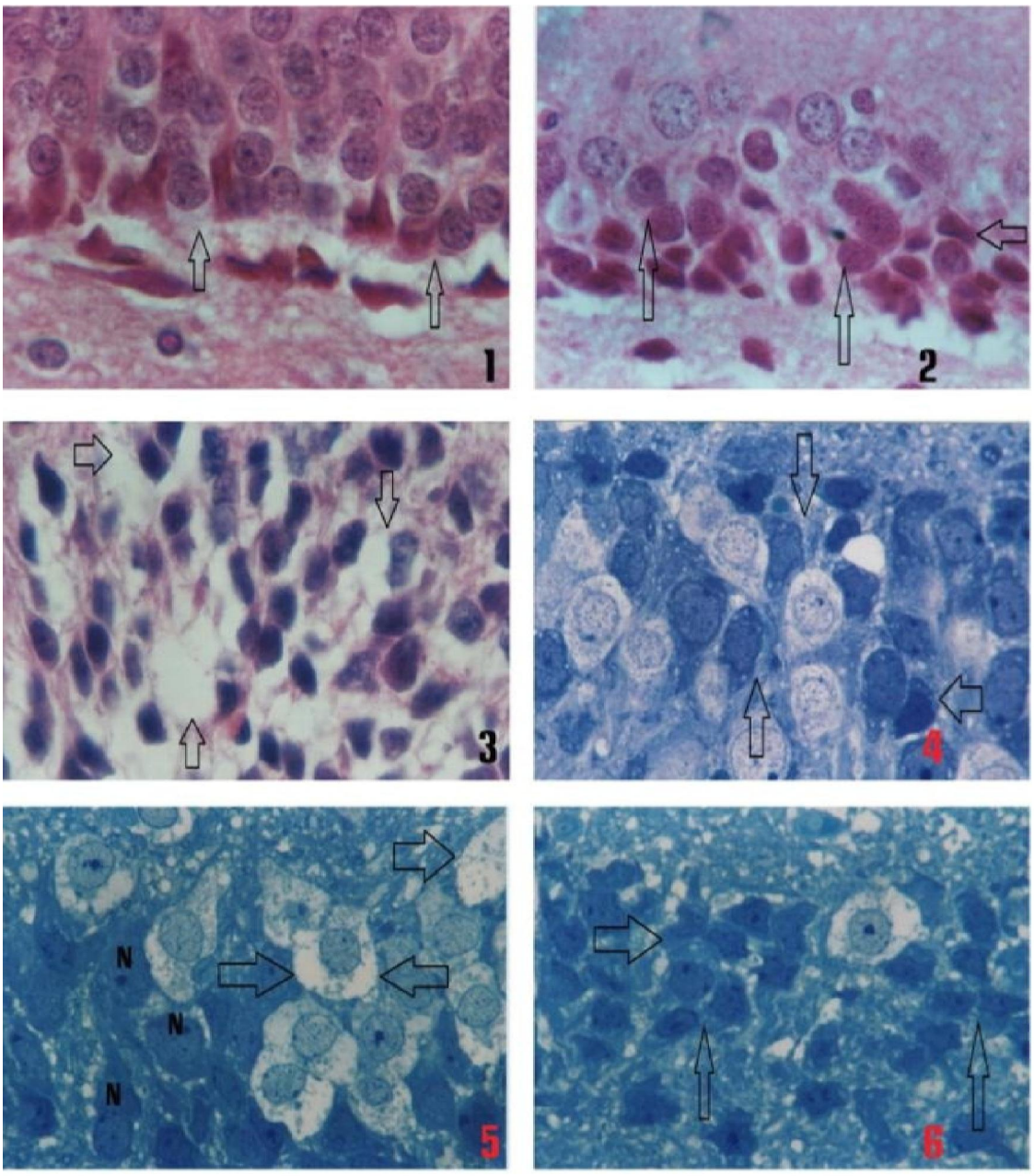

PLATE 2

Fig. 1: A photomicrograph of a section in dentate gyrus of the control 12 months - aged group showing; the intense staining of granule cell bodies in the deeper parts of the granular cell layer (arrows). H.&E. X 1000

Fig. 2: A photomicrograph of a section in dentate gyrus of the control 18 months- aged group showing; many granule cells with darkly stained nucleus and cytoplasm (arrows). H.&E. X 1000

Fig. 3: A photomicrograph of a section in dentate gyrus of the control 24 months - aged group showing; dark staining of most neurons in the granule cell layer and large empty spaces among granule cell bodies (arrows). H.&E. X1000

Fig. 4: A photomicrograph of a semithin section in dentate gyrus of the control 12 months - aged group showing; some granule cell bodies appear darkly stained with irregular outlines (arrows). Toluidine blue X1000

Fig. 5: A photomicrograph of a semithin section in dentate gyrus of the control 18 months - aged group showing; some granule cell bodies appear darkly stained with irregular outlines (N) and large vacuolated areas(arrows). Toluidine blue X1000

Fig. 6: A photomicrograph of a semithin section in dentate gyrus of the control 24 months - aged group showing; most of the granule cell bodies appear darkly stained with irregular outlines (arrows). Toluidine blue X1000

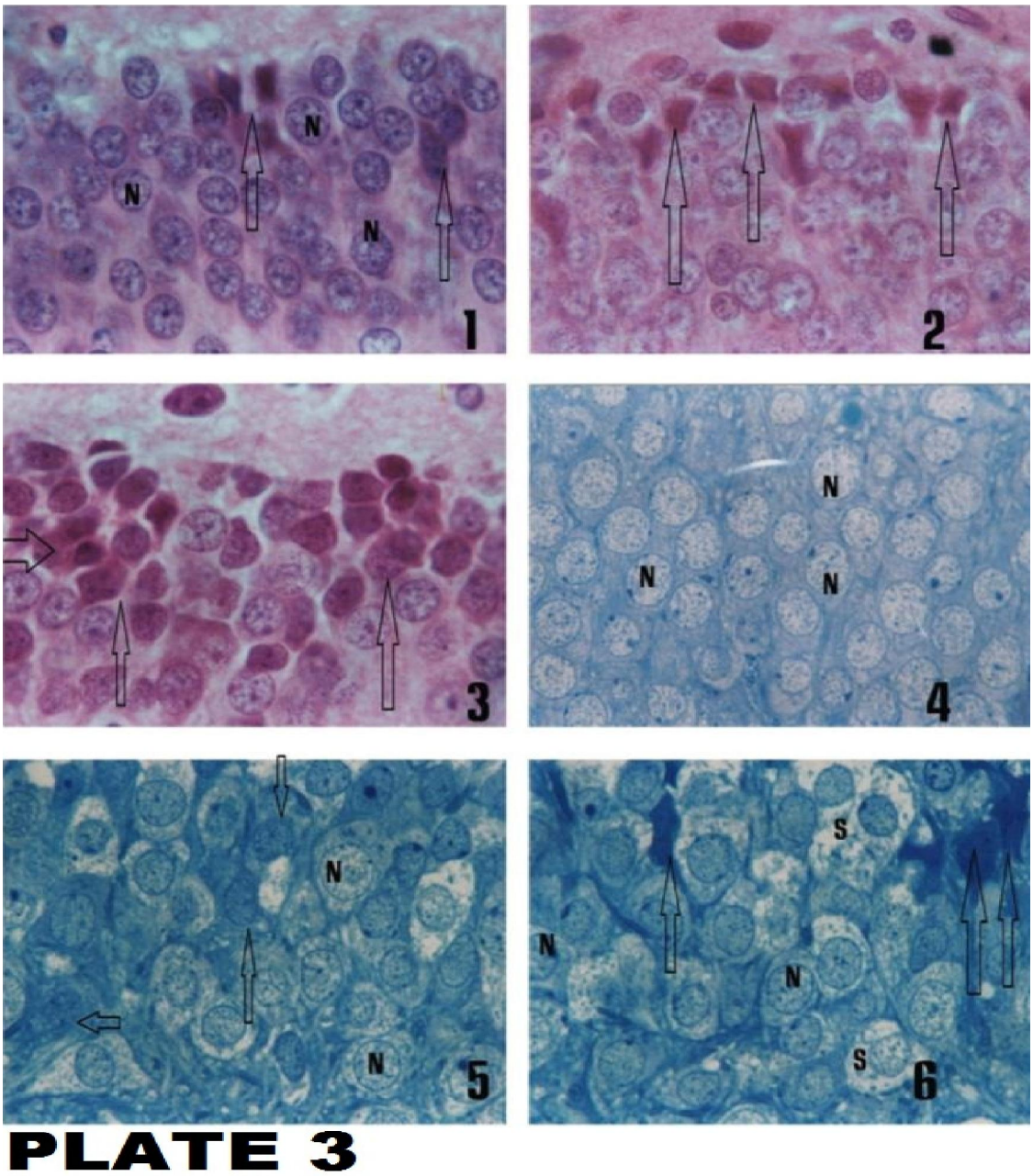

PLATE 3

Fig. 1: A photomicrograph of a section in dentate gyrus of the Antox treated 12 months – aged group showing; the normal appearance of most granule cells (N) with few deeply stained irregular granule cells (arrows). H. & E. X 1000

Fig. 2: A photomicrograph of a section in dentate gyrus of the Antox treated 18 months – aged group showing; the normal appearance of many granule cells with few stained irregular granule cells (arrows). H.&E. X 1000

Fig. 3: A photomicrograph of a section in dentate gyrus of the Antox treated 24 months – aged group showing; less empty spaces appear among granule cell bodies as compared with the control group of the same age group. H. & E. X1000

Fig. 4: A photomicrograph of a semithin section in dentate gyrus of the Antox treated 12 months – aged group showing; most of the granule cells have rounded pale nuclei with dense nucleoli much similar to the control group of the same age group.Toluidine blue X1000

Fig. 5: A photomicrograph of a semithin section in dentate gyrus of the Antox treated 18 months – aged group showing; many of the granule cells have rounded pale nuclei with dense nucleoli surrounded by pale cytoplasm. Few granule cells appear more darkly stained. Toluidine blue X1000

Fig. 6: A photomicrograph of a semithin section in dentate gyrus of the Antox treated 24 months – aged group showing; some of the granule cell bodies appear with rounded nuclei, empty spaces appear among granule cell bodies (arrows) and some darkly stained with irregular outlines granule cells. Toluidine blue X1000

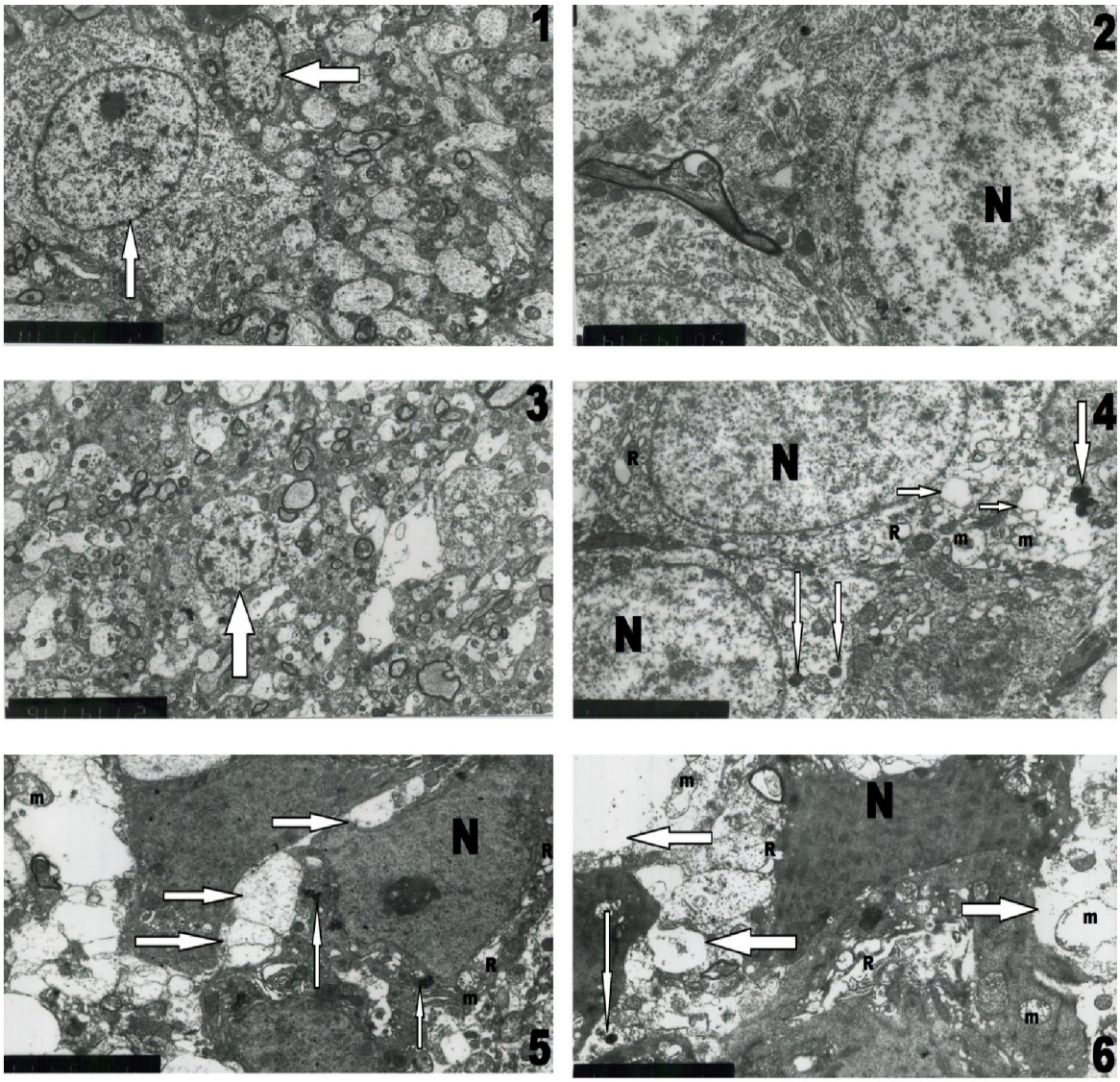

PLATE 4

Fig. 1: An electron micrograph of a section in dentate gyrus of the control adult group showing; a part of the molecular layer in which a displaced granule cell (left vertical arrow) seen the molecular layer. Beside it, a glial cell of smaller nucleus with scanty cytoplasm could be seen (right horizontal arrow). (X 2700).

Fig. 2: An electron micrograph of a section in dentate gyrus of the control adult group showing; a part of the granule cell layer in which the granule cells have pale rounded nuclei with finely dispersed chromatin (N). (X 5000).

Fig. 3: An electron micrograph of a section in dentate gyrus of the control adult group showing; a part of the polymorphic layer in which numerous nerve fibers. A glial cell is also seen (arrow). (X 2700).

Fig. 4: An electron micrograph of a section in dentate gyrus of the control 12 months - aged group showing; a part of the granular cell layer in which there is different electron density of granule cell bodies(N), lipofuscin pigment (vertical arrows), vacuoles (horizontal arrows), dilated short segments of rER (R) and distorted mitochondria (m). (X 4000).

Fig. 5: An electron micrograph of a section in dentate gyrus of the control 18 months - aged group showing; a part of the granular cell layer in which there is dark granule cell bodies (N), dilated short segments of rER (R), lipofuscin pigment (vertical arrows) and distorted mitochondria (m) and organelle - free areas among the granule cell bodies (horizontal arrows). (X 4000).

Fig. 6: An electron micrograph of a section in dentate gyrus of the control 24 months - aged group showing; a part of the granular cell layer in which there is marked increase in electron density of granule cells(N), dilated short segments of rER (R), lipofuscin pigment in their cytoplasm (vertical arrow) and distorted mitochondria (m)and many organelle - free areas among the granule cell bodies (horizontal arrows). (X5000).

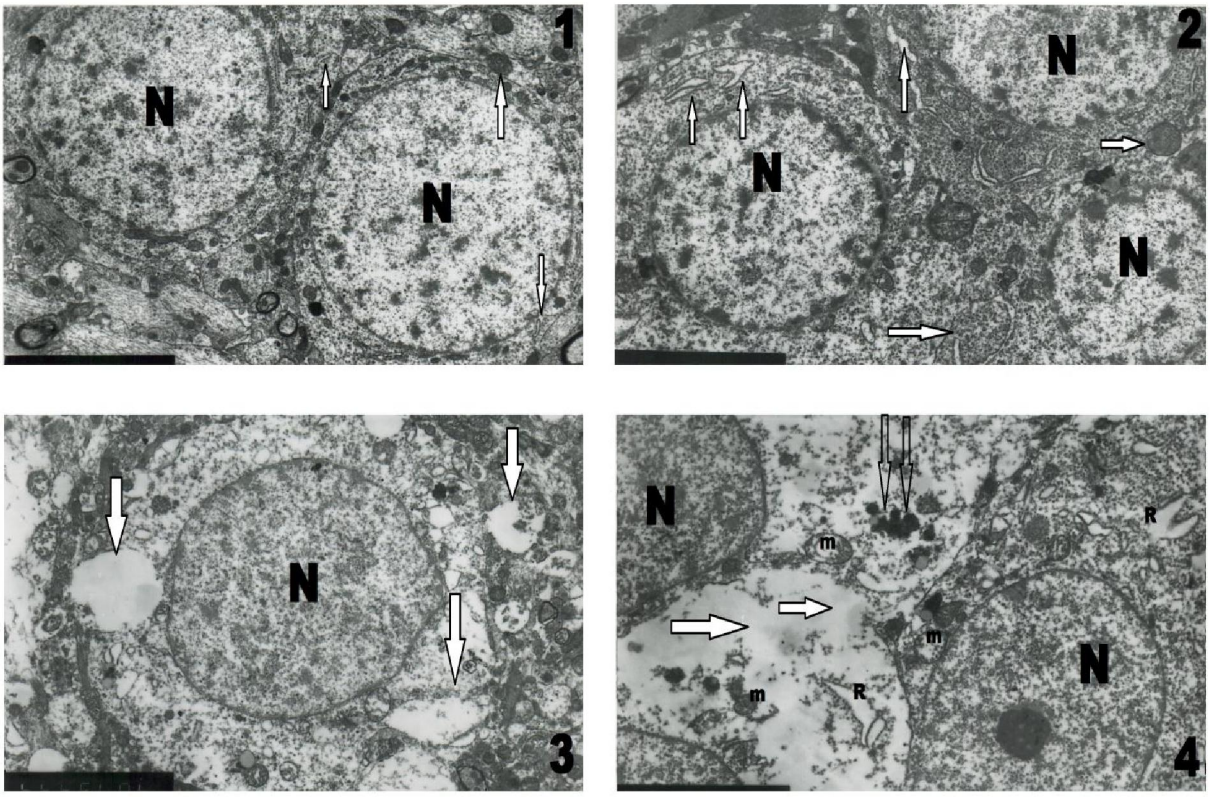

PLATE 5

Fig. 1: An electron micrograph of a section in dentate gyrus of the Antox treated 12 months – aged group showing; a relatively normal shape and electron density of nuclei of granule cells (N)and normal appearance of neuronal organelles (arrows). (X 4000).

Fig. 2: An electron micrograph of a section in dentate gyrus of the Antox treated 18 months – aged group showing; some of granule cells nuclei (N), dilated rER cisternae (vertical arrows) but little distortion of mitochondria (horizontal arrows). (X 4000).

Fig. 3: An electron micrograph of a section in dentate gyrus of the Antox treated 18 months – aged group showing; small organelle – free areas in the cytoplasm of granule cells (arrows). (X 4000).

Fig. 4: An electron micrograph of a section in dentate gyrus of the Antox treated 24 months – aged group showing; some of granule cells nuclei (N), dilated rER cisternae (R), lipofuscin pigment (vertical arrows) distortion of mitochondria (m) and organelle – free areas in the cytoplasm of granule cells (horizontal arrows). (X 4000).

Hawazenet and Maisaa, (2007)studied the effect of Antox on kidney of albino rats. They found that the antioxidant Antox led to an improvement in both histological and biochemical alteration of rats induced by toxic herbicide paraquat**. Sonali*et al.*,(2006)** stated that, the ameliorative potential of selenium revealed a positive role of selenium, especially when Se preceded As_2O_3 treatment in either *in vitro* or *in vivo*.

The noticeable reduction in the age-associated histological changes which was observed in the treated animals was less compared with those of the control aged animals. This observation reflects the protective effect of Antox against the free radical attack of mitochondrial membranes and mitochondrial DNA. These results correlates with that made by **Amal and Mona,(2009)** who reported that rats treated with Antox revealed an improvement in histopathological alteration after 3 weeks and 6 weeks. This proved the effectiveness of Antox that attributed to its antioxidant properties.

The present study showed that the protective effect of Antox against all these age-associated histological changes was noticeable in all age groups of treated animals, but to a varying degree with more protection being observed at the younger age groups. It is also noticeable that the preventive **effect of antioxidant** on age-associated histological changes was obvious **but this** prevention varied in degree according to the age group. Thus, **the** preventive

effect of antioxidants was best in the middle age group, **which** restored histological structure much similar to the adult group. The prevention was less in the early old group so that not all the neurons restored the normal shape and staining intensity with normal ultrastructure. Furthermore, in the oldest group, still some of the neurons showed some degenerative changes. These observations show a positive correlation between better restoration of neuronal structure and earlier treatment with antioxidants. This can be explained by that, at middle age, there were early reversible changes before the administration of antioxidants which can restore these changes and prevent the occurrence of any other degenerative changes. But, later in early old and oldest old groups, more irreversible degenerative changes had developed before the administration of antioxidants **(Murray and Lynch, 1998 a&b)**.

The incomplete prevention of all degenerative changes in older age groups by Antox may be due to the contribution of otherfactors, besides oxidative damage, to the aging process as the process ofaging is known to be a multifactorial process. Therefore, neuroendocrine factors, immunological factors, or vascular factors can contribute toa varying degree in the aging process. However, the marked noticeable improvement in age-associated histological degenerative changes as compared with the control of the same ages, signify the high contribution of tree radical-mediated oxidative damage to be the major causative factor of brain aging. Therefore, antioxidant therapies are being promoted to enhance mental functions and delay cognitive losses with aging. An increasing number of physicians are also recommending antioxidant therapies, for subjects with Alzheimer's disease and other neurodegenerative disorders (Von Arnim***et al.,*** **2012)**.

Conclusion

The present study showed that the age - associated histological changes may be the basis for the age associated functional changes of the dentate gyrusof the hippocampal formation, which may be manifest in elderlypeople by disturbances in motor coordination and declines. The present study also demonstrates the effectiveness of the combination antioxidants (Antox) in reducing the age-related histological changes in the dentate gyrus of the hippocampal formation. So, it is recommended to investigate its use in age-related neurodegenerative disorders human, for improvement of learning and memory during aging.

Corresponding authors
Mohammed. H. Mohammed
Department of Anatomy and Embryology, Faculty of Medicine, Assiut University.

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